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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

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For: METHODS FOR SYNTHESIS OF OLIGONUCLEOTIDES

MS Appeal Brief - Patents
Commissioner for Patents
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APPENDIX A TO APPELLANT'S BRIEF

1. A method for the preparation of a linear phosphorus-linked oligomer comprising the steps of:

(a) providing a solid support;

(b) attaching a 5'-O-protected nucleoside to the solid support;

(c) deprotecting the 5'-hydroxyl of the nucleoside with a deprotecting reagent comprising a protic acid in a solvent to deprotect the 5'-hydroxyl of the nucleoside, wherein the solvent consists essentially of an aromatic solvent, an alkyl aromatic solvent, a halogenated aromatic solvent, a halogenated alkyl aromatic solvent, or an aromatic ether solvent;

(d) reacting the deprotected 5'-hydroxyl with an 5'-protected activated phosphorus compound to produce a covalent linkage therebetween;

(e) oxidizing or sulfurizing the covalent linkage to form a phosphodiester, phosphorothioate, phosphorodithioate or H-phosphonate linkage;

(f) repeating steps c through e at least once for subsequent couplings of additional activated phosphorus compounds, to produce the completed phosphorus-linked oligomer; and

(g) cleaving the oligomer from the solid support;

wherein steps (b) through (f) are performed with an automated device;

wherein said oligomer is a linear oligomer.

2. The method of claim 1 further comprising the step of capping remaining reactive sites with a solution containing a capping reagent either immediately before said covalent linkage is oxidized or sulfurized or immediately after said covalent linkage is oxidized or sulfurized.

3. The method of claim 1 wherein the oxidation or sulfurization step is performed after each iteration of steps (c) and (d).

4. The method of claim 1 wherein the oxidation or sulfurization step is performed after the final iteration of steps (c) and (d).

5. The method of claim 1 wherein the solvent in step (c) is an aromatic solvent, an alkyl aromatic solvent, or an aromatic ether.

6. The method of claim 1 wherein the solvent in step (c) is selected from the group consisting of o-xylene, m-xylene, p-xylene, mesitylene, and diphenyl ether.

7. The method of claim 6 wherein the solvent in step (c) is selected from the group consisting of benzene, toluene, o-xylene, m-xylene, and p-xylene.

8. The method of claim 7 wherein the solvent in step (c) is toluene.

9. The method of claim 1 wherein the solvent in step (c) is a halogenated aromatic solvent or a halogenated alkyl aromatic solvent.

10. The method of claim 9 wherein the solvent in step (c) is chlorobenzene or benzotrifluoride.

11. The method of claim 1 wherein the activated phosphorus compound is selected from the group consisting of an activated mononucleotide, an activated dinucleotide, and an activated polynucleotide.

12. The method of claim 1 wherein the activated phosphorus compound is a 5'-protected nucloside phosphoramidite or a 5'-protected activated H-phosphonate nucleoside.

13. The method of claim 1 wherein the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is independently selected from the group consisting of trityl, monomethoxy trityl, dimethoxytrityl, trimethoxytrityl, 2-chlorotrityl, 1,1-dianisyl-2,2,2-triclhoroethyl (DATE), 4,4',4"-tris(benzoyloxyphenyl)methyl (TBTr) , 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX).

14. The method of claim 13 wherein the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is independently selected from the group consisting of trityl, monomethoxy trityl, dimethoxy trityl, 9-phenylxanthine-9-yl (Pixyl) [or] and 9-(p-methoxyphenyl)xanthine-9-yl.

15. The method of claim 14 wherein the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is dimethoxytrityl.

16. The method of claim 1 wherein the phosphorus-linked oligomer is selected from the group consisting of a phosphodiester, a phosphorothioate phosphorodithioate, and a H-phosphonate oligonucleotide.

17. The method of claim 1 wherein the protic acid is selected from the group consisting of formic acid, acetic acid, chloroacetic acid, dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid, benzenesulfonic acid, toluenesulfonic acid, and phenylphosphoric acid.

18. The method of claim 1 wherein the solvent in step (c) further comprises an additive.

19. The method of claim 18 wherein the additive to the solvent in step (c) is an alcohol.

20. The method of claim 19 wherein the alcohol additive to the solvent in step (c) is selected from the group consisting of from 0% to about 30% methanol, ethanol, 2-propanol, t-butyl alcohol, t-amyl alcohol, benzyl alcohol, 1,1,1,3,3,3-hexafluoro-2-propanol, and mixtures thereof.

21. A method for the preparation of a linear phosphorus-linked oligomer comprising the steps of:

(a) providing a solid support;

(b) attaching a 5'-O-protected nucleoside to the solid support;

(c) contacting the protected 5'-hydroxyl of the nucleoside with a deprotecting reagent comprising a protic acid in a solvent to deprotect the 5'-hydroxyl of the

nucleoside, wherein the solvent consists essentially of an aromatic solvent, an alkyl aromatic solvent, a halogenated aromatic solvent, a halogenated alkyl aromatic solvent, or an aromatic ether solvent;

(d) reacting the deprotected 5'-hydroxyl with a 5'-protected activated phosphite compound to produce a phosphite linkage;

(e) oxidizing or sulfurizing the phosphite linkage to form a phosphodiester, phosphorothioate, or phosphorodithioate linkage;

(f) repeating steps c through e at least once for subsequent couplings of additional activated phosphite compounds, to produce the completed phosphorus-linked oligomer; and

(g) cleaving the oligomer from the solid support;

wherein steps (b) through (f) are performed with an automated device;

wherein said oligomer is a linear oligomer.

22. The method of claim 21 further comprising the step of capping remaining reactive sites with a solution containing a capping reagent either immediately before said covalent linkage is oxidized or sulfurized or immediately after said covalent linkage is oxidized or sulfurized.

23. The method of claim 21 wherein the solvent in step (c) is selected from the group consisting of an aromatic solvent, an alkyl aromatic solvent, and an aromatic ether.

24. The method of claim 23 wherein the solvent in step (c) is selected from the group consisting of benzene, toluene, benzonitrile, o-xylene, m-xylene, p-xylene, mesitylene, and diphenyl ether.

25. The method of claim 24 wherein the solvent in step (c) is selected from the group consisting of benzene, toluene, o-xylene, m-xylene, and p-xylene.

26. The method of claim 25 wherein the solvent in step (c) is toluene.

27. The method of claim 21 wherein the solvent in step (c) is a halogenated aromatic solvent or a halogenated alkyl aromatic solvent.

28. The method of claim 27 wherein the solvent in step (c) is chlorobenzene or benzotrifluoride.

29. The method of claim 21 wherein the activated phosphite compound is selected from the group consisting of a mononucleotide phosphoramidite, a dinucleotide phosphoramidite, and a polynucleotide phosphoramidite.

30. The method of claim 21 wherein the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is independently selected from the

group consisting of trityl, monomethoxy trityl, dimethoxytrityl, trimethoxytrityl, 2-chlorotrityl, 1,1-dianisyl-2,2,2-triclhoroethyl (DATE) , 4,4',4"-tris(benzoyloxyphenyl)methyl (TBTr), 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX).

31. The method of claim 30 wherein the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is independently selected from the group consisting of trityl, monomethoxy trityl, dimethoxy trityl, 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl.

32. The method of claim 31 wherein the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is dimethoxytrityl.

33. The method of claim 21 wherein the phosphorus-linked oligomer is selected from the group consisting of a phosphodiester, phosphorothioate and a phosphorodithioate oligonucleotide.

34. The method of claim 21 wherein the protic acid is selected from the group consisting of formic acid, acetic acid, chloroacetic acid, dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid, benzenesulfonic acid, toluenesulfonic acid, and phenylphosphoric acid.

35. The method of claim 21 wherein the solvent in step (c) further comprises an additive.

36. The method of claim 35 wherein the additive to the solvent in step (c) is an alcohol.

37. The method of claim 36 wherein the alcohol additive to the solvent in step (c) is selected from the group consisting of from 0% to about 30% methanol, ethanol, 2-propanol, t-butyl alcohol, t-amyl alcohol, benzyl alcohol, 1,1,1,3,3,3-hexafluoro-2-propanol, [or a mixture] and mixtures thereof.

38. The method of claim 22 wherein the solvent in step (c) is selected from the group consisting of benzene, toluene, benzonitrile, o-xylene, p-xylene, mesitylene, and diphenyl ether; the activated phosphite compound is selected from the group consisting of a mononucleotide phosphoramidite, a dinucleotide phosphoramidite, and a polynucleotide phosphoramidite; the protecting group of the 5'-O-protected nucleoside and the 5'-protected activated phosphite compound is dimethoxytrityl; the phosphorus linked oligomer is selected from the group consisting of a phosphodiester, phosphorothioate and a phosphorodithioate oligonucleotide; and the protic acid is dichloroacetic acid.

39. The method of claim 38 wherein the solvent in step (c) is toluene.

40. The method of claim 39 wherein the activated phosphite compound is a mononucleotide phosphoramidite.

41. The method of claim 1 wherein the 5'-protected activated phosphorus compound is a 5'-protected activated H-phosphonate compound; and the phosphorus-linked oligomer is an H-phosphonate oligonucleotide.

42. A method for the preparation of a linear phosphorus-linked oligomer comprising the steps of:

(a) providing a solid support;

(b) attaching a 5'-O-protected nucleoside to the solid support;

(c) deprotecting the 5'-hydroxyl of the nucleoside with a deprotecting reagent comprising dichloroacetic acid in toluene;

(d) reacting the deprotected 5'-hydroxyl with an 5'-protected activated phosphorus compound to produce a covalent linkage therebetween;

(e) oxidizing or sulfurizing the covalent linkage to form a phosphodiester, phosphorothioate, phosphorodithioate or H-phosphonate linkage;

(f) repeating steps c through e at least once for subsequent couplings of additional activated phosphorus compounds, to produce the completed phosphorus-linked oligomer; and

(g) cleaving the oligomer from the solid support;

wherein steps (b) through (f) are performed with an automated device;

wherein said oligomer is a linear oligomer.